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Term: L1 and bacter$3 flora

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DATE: Thursday, April 03, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L2	L1 and bacter$3 flora	1	L2
L1	6287769.pn.	2	L1

END OF SEARCH HISTORY

10/069,977

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L12	l9 and apparatus	6	L12
L11	L10 and apparatus	0	L11
L10	L9 and elestrophoresis	0	L10
L9	l1 and (DNA near10 hybridiz$5)	25	L9
L8	bacter$3 flora near5 DNA	1	L8
L7	bacter$3 near5 DNA	12119	L7
L6	L5 and apparatus	5	L6
L5	L4 and probe$1	23	L5
L4	L3 and electrophoresis	27	L4
L3	l1 and pcr	42	L3
L2	Bacter$3 flora near5 PCR	1	L2
L1	bacterial flora	984	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 6 of 6 returned.

☑ 1. 5750363 . 19 May 97; 12 May 98. Method for determining the antimicrobial agent sensitivity of a nonparaffinophilic microorganism and an associated apparatus. Ollar; Robert-A., et al. 435/29; 422/50 422/68.1 435/283.1 435/287.1 435/30 435/32 435/34 435/36 435/4 435/42 435/848 435/849 435/852 435/879 435/882 435/883 435/885. C12Q001/02 C12Q001/24 C12Q001/18 C12Q001/14.

☐ 2. 5580787 . 13 Aug 92; 03 Dec 96. Cloning vector for use in lactic acid bacteria. Wessels; Stephen, et al. 435/320.1; 424/93.45 426/34 435/139 435/252.3 435/69.1. C12N015/09 C12N015/63 C12N015/74 C12P007/56.

☐ 3. 5326857 . 29 Aug 91; 05 Jul 94. ABO genotyping. Yamamoto; Fumi-ichiro, et al. 536/23.2; 435/320.1 435/367 536/23.1. C07H021/02 C12N015/70 C12N005/10.

☐ 4. 5116754 . 04 Oct 90; 26 May 92. Separation of bacteria from organic matter. Fraser; Ann D. E., et al. 435/252.1; 210/499 210/609 210/611 435/252.4 435/261 435/287.1 435/308.1. C12N001/12 C12M001/00.

☐ 5. 5068191 . 31 Aug 89; 26 Nov 91. Purified histo-blood group A glycosyltransferase and antibodies thereto. Clausen; Henrik, et al. 530/388.26; 435/183 435/193 435/343 530/386 530/395. C12N009/12 C12N005/02 A61K035/14 C12P021/08.

☐ 6. WO 200120032 A1 EP 1215285 A1 JP 2001149073 A . Amplification of specific sequences from bacterial DNA in a biological sample, for diagnostic analysis of intestinal flora. INOUE, T. C12M001/00 C12M001/38 C12N015/09 C12Q001/02 C12Q001/68 G01N033/50.

Generate Collection | Print

Term	Documents
APPARATUS.DWPI,EPAB,JPAB,USPT.	2737142
APPTS.DWPI,EPAB,JPAB,USPT.	556760
(9 AND APPARATUS).USPT,JPAB,EPAB,DWPI.	6
(L9 AND APPARATUS).USPT,JPAB,EPAB,DWPI.	6

Previous Page Next Page

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FILE 'HOME' ENTERED AT 12:57:12 ON 03 APR 2003

=> file medline caplus biosis
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.63       0.63

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=> s bater### (10A) flora (10a)DNA
L1            0 BATER### (10A) FLORA (10A) DNA

=> s bacter###(10a)flora(10a)DNA
L2           35 BACTER###(10A) FLORA(10A) DNA

=> s l2 and CR
L3            0 L2 AND CR

=> s l2 and PCR
L4            9 L2 AND PCR

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5            5 DUP REM L4 (4 DUPLICATES REMOVED)

=> d l5 1-5 bib ab kwic

L5   ANSWER 1 OF 5     MEDLINE                              DUPLICATE 1
AN   2003059332     MEDLINE
DN   22423434   PubMed ID: 12534811
TI   Employment of broad-range 16S rRNA PCR to detect aetiological
     agents of infection from clinical specimens in patients with acute
     meningitis--rapid separation of 16S rRNA PCR amplicons without
     the need for cloning.
AU   Xu J; Millar B C; Moore J E; Murphy K; Webb H; Fox A J; Cafferkey M; Crowe
     M J
CS   Northern Ireland Public Health Laboratory, Department of Bacteriology,
     Belfast City Hospital, Belfast, UK.
SO   JOURNAL OF APPLIED MICROBIOLOGY, (2003) 94 (2) 197-206.
     Journal code: 9706280. ISSN: 1364-5072.
CY   England: United Kingdom
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200303
ED   Entered STN: 20030207
     Last Updated on STN: 20030307
     Entered Medline: 20030306
AB   AIMS: The aim of this study was to develop a polyacrylamide gel
     electrophoresis (PAGE) method for the rapid separation of 16S rRNA
     PCR amplicons from aetiological agents of acute meningitis.
```

METHODS AND RESULTS: Blood samples from 40 patients with suspected acute meningococcal meningitis were examined for the presence of causal agents, including Neisseria meningitidis employing two methods: (i) broad-range 16S rRNA **PCR** in conjunction with PAGE and automated sequencing and (ii) species-specific **PCR** employing ABI TaqMan technology for N. meningitidis. Analysis of clinical specimens employing 16S rRNA **PCR** yielded 33/40 (82.5%) positive for the presence of bacterial DNA. Species-specific **PCR** yielded 30/40 (75%) clinical specimens positive for N. meningitidis. Prior to separation by PAGE, only 6/33 (18.2%) amplicons were able to be identified by sequence analysis, the remaining amplicons (n=27) did not yield an identification due to the presence of mixed 16S rRNA **PCR** amplicons. Following separation, amplicons were re-amplified and sequenced, yielding 24/27 (88.9%) positive for N. meningitidis and three specimens positive for Acinetobacter sp., Staphylococcus aureus and Streptococcus pneumoniae. One specimen was positive for both N. meningitidis and Streptococcus spp. and another specimen was positive for N. meningitidis and Pseudomonas sp., by broad-range **PCR**. Seven clinical specimens were negative for N. meningitidis and other eubacteria using both detection techniques. CONCLUSIONS: Clinical specimens including blood and cerebrospinal fluid from patients with suspected acute **bacterial** meningitis, may become contaminated with commensal skin **flora**, resulting in difficulties in downstream sequencing of pathogen plus contaminant **DNA**. This study allows for the rapid separation of amplified pathogen from contaminant DNA. SIGNIFICANCE AND IMPACT OF STUDY: This study demonstrated the usefulness of the rapid separation of multiple 16S rRNA **PCR** amplicons using a combination of PAGE and automated sequencing, without the need of cloning. Adoption of this technique is therefore proposed when trying to rapidly identify pathogens in clinical specimens employing broad-range 16S rRNA **PCR**.

TI Employment of broad-range 16S rRNA **PCR** to detect aetiological agents of infection from clinical specimens in patients with acute meningitis--rapid separation of 16S rRNA **PCR** amplicons without the need for cloning.

AB . . . aim of this study was to develop a polyacrylamide gel electrophoresis (PAGE) method for the rapid separation of 16S rRNA **PCR** amplicons from aetiological agents of acute meningitis. METHODS AND RESULTS: Blood samples from 40 patients with suspected acute meningococcal meningitis were examined for the presence of causal agents, including Neisseria meningitidis employing two methods: (i) broad-range 16S rRNA **PCR** in conjunction with PAGE and automated sequencing and (ii) species-specific **PCR** employing ABI TaqMan technology for N. meningitidis. Analysis of clinical specimens employing 16S rRNA **PCR** yielded 33/40 (82.5%) positive for the presence of bacterial DNA. Species-specific **PCR** yielded 30/40 (75%) clinical specimens positive for N. meningitidis. Prior to separation by PAGE, only 6/33 (18.2%) amplicons were able. . . by sequence analysis, the remaining amplicons (n=27) did not yield an identification due to the presence of mixed 16S rRNA **PCR** amplicons. Following separation, amplicons were re-amplified and sequenced, yielding 24/27 (88.9%) positive for N. meningitidis and three specimens positive for. . . for both N. meningitidis and Streptococcus spp. and another specimen was positive for N. meningitidis and Pseudomonas sp., by broad-range **PCR**. Seven clinical specimens were negative for N. meningitidis and other eubacteria using both detection techniques. CONCLUSIONS: Clinical specimens including blood and cerebrospinal fluid from patients with suspected acute **bacterial** meningitis, may become contaminated with commensal skin **flora**, resulting in difficulties in downstream sequencing of pathogen plus contaminant **DNA**. This study allows for the rapid separation of amplified pathogen from contaminant DNA. SIGNIFICANCE AND IMPACT OF STUDY: This study demonstrated the usefulness of the rapid separation of multiple 16S rRNA **PCR** amplicons using a combination of PAGE and automated sequencing, without the need of cloning. Adoption of this technique is therefore proposed when trying to rapidly

identify pathogens in clinical specimens employing broad-range 16S rRNA **PCR**.

L5 ANSWER 2 OF 5 MEDLINE
AN 2003147330 IN-PROCESS
DN 22549487 PubMed ID: 12662383
TI Profiling and Identification of Eubacteria in the Stomach of Mongolian Gerbils With and Without Helicobacter pylori Infection.
AU Sun Yi-Qian; Monstein Hans-Jurg; Nilsson Lennart E; Petersson Fredrik; Borch Kurt
CS Division of Surgery, Department of Biomedicine and Surgery; Molecular Biology Laboratory - LMO; Clinical Microbiology - IMK; Faculty of Health Sciences, University of Linkoping, and Pathology Research Department, Ryhov Hospital, Jonkoping, Sweden.
SO HELICOBACTER, (2003 Apr) 8 (2) 149-57.
Journal code: 9605411. ISSN: 1083-4389.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20030331
Last Updated on STN: 20030331
AB BACKGROUND: Mongolian gerbils are frequently used to study Helicobacter pylori-induced gastritis and its consequences. The presence of an indigenous bacterial flora with suppressive effect on H. pylori may cause difficulties with establishing this experimental model. AIM: The aim of the present study was to determine bacterial profiles in the stomach of Mongolian gerbils with and without (controls) H. pylori infection. METHODS: Gastric tissue from H. pylori ATCC 43504 and CCUG 17874 infected and control animals were subjected to microbial culturing and histology. In addition, gastric mucosal samples from H. pylori ATCC 43504 infected and control animals were analyzed for bacterial profiling by temporal temperature gradient gel electrophoresis (TTGE), cloning and pyrosequencing of 16S rDNA variable V3 region derived **PCR** amplicons. RESULTS: Oral administration of H. pylori ATCC 43504, but not CCUG 17874, induced colonization and gastric inflammation in the stomach of Mongolian gerbils. Temporal temperature gradient gel electrophoresis (TTGE) and partial 16S rDNA pyrosequencing revealed the presence of **DNA** representing a mixed **bacterial flora** in the stomach of both H. pylori ATCC 43504 infected and control animals. In both cases, lactobacilli appeared to be dominant. CONCLUSION: These findings suggest that indigenous bacteria, particularly lactobacilli, may have an impact on the colonization and growth of H. pylori strains in the stomach of Mongolian gerbils.
AB . . . for bacterial profiling by temporal temperature gradient gel electrophoresis (TTGE), cloning and pyrosequencing of 16S rDNA variable V3 region derived **PCR** amplicons. RESULTS: Oral administration of H. pylori ATCC 43504, but not CCUG 17874, induced colonization and gastric inflammation in the stomach of Mongolian gerbils. Temporal temperature gradient gel electrophoresis (TTGE) and partial 16S rDNA pyrosequencing revealed the presence of **DNA** representing a mixed **bacterial flora** in the stomach of both H. pylori ATCC 43504 infected and control animals. In both cases, lactobacilli appeared to be. . .

L5 ANSWER 3 OF 5 MEDLINE DUPLICATE 2
AN 2002111595 MEDLINE
DN 21831570 PubMed ID: 11843027
TI Identification of Helicobacter pylori DNA in human cholesterol gallstones.
AU Monstein H J; Jonsson Y; Zdolsek J; Svanvik J
CS Molecular Biology Laboratory-LMO, University Hospital, Linkoping, Sweden.. hanmo@ibk.liu.se
SO SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY, (2002 Jan) 37 (1) 112-9.
Journal code: 0060105. ISSN: 0036-5521.

CY Norway
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200207
ED Entered STN: 20020215
Last Updated on STN: 20020727
Entered Medline: 20020726
AB BACKGROUND: The gallbladder mucosa secretes hydrogen ions and is covered by mucus. The environmental conditions for bacterial colonization are similar to those in the stomach. Gallbladder stones often contain DNA from enteric bacteria, but no compelling evidence demonstrates that Helicobacter spp. have been present. The aim of this study was to establish bacterial DNA profiles in cholesterol gallstones with special reference to Helicobacter pylori. METHODS: Cholesterol gallstones from 20 patients were subjected to polymerase chain reaction, bacterial profiling by temporal temperature gradient gel electrophoresis, automated DNA sequencing, and Southern blot analysis using a Helicobacter sp. specific primer. A nested ureI-**PCR** assay was used to discriminate between gastric and non-gastric H. pylori. RESULTS: TTGE, partial 16S rDNA sequencing, and hybridization analysis revealed the presence of **DNA** presumably representing a mixed **bacterial flora** in cholesterol gallstones, including H. pylori in the gallstone centres in 11 out of 20 patients. In three cases, the urel-**PCR** assay revealed non-gastric H. pylori. CONCLUSIONS: These data support the presence of DNA from a mixed bacterial population, including H. pylori in cholesterol gallstones, reflecting either that H. pylori is an indigenous part of a flora in the stone-containing gallbladder or, alternatively, that H. pylori colonization in the biliary tract predisposes to cholesterol gallstone formation.
AB . . . temporal temperature gradient gel electrophoresis, automated DNA sequencing, and Southern blot analysis using a Helicobacter sp. specific primer. A nested ureI-**PCR** assay was used to discriminate between gastric and non-gastric H. pylori. RESULTS: TTGE, partial 16S rDNA sequencing, and hybridization analysis revealed the presence of **DNA** presumably representing a mixed **bacterial flora** in cholesterol gallstones, including H. pylori in the gallstone centres in 11 out of 20 patients. In three cases, the urel-**PCR** assay revealed non-gastric H. pylori. CONCLUSIONS: These data support the presence of DNA from a mixed bacterial population, including H.. . .

L5 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS
AN 2000:665205 CAPLUS
DN 134:173678
TI Comparison of the oral bacterial flora in saliva from a healthy subject and two periodontitis patients by sequence analysis of 16S rDNA libraries
AU Sakamoto, Mitsuo; Umeda, Makoto; Ishikawa, Isao; Benno, Yoshimi
CS Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Saitama, 351-0198, Japan
SO Microbiology and Immunology (2000), 44(8), 643-652
CODEN: MIIMDV; ISSN: 0385-5600
PB Center for Academic Publications Japan
DT Journal
LA English
AB The oral bacterial flora in the saliva from two patients with periodontitis and from a periodontally healthy subject were compared using a sequence anal. of 16S rDNA libraries without cultivation. 16S rDNAs were amplified from salivary DNA by **PCR** and cloned. Randomly selected clones were partially sequenced. On the basis of sequence similarities, the clones were classified into several clusters corresponding to the major phylum of the domain Bacteria. The major phylum in the libraries was the low G + C Gram-pos. bacteria. There was no clonal sequence affiliated with periodontopathic bacteria in the

salivary sample from the healthy subject, while a no. of periodontal pathogens such as Campylobacter rectus, Prevotella intermedia, Porphyromonas gingivalis and Treponema socranskii were detected in the salivary samples from the patients with periodontitis. In addn., a no. of previously uncharacterized and uncultured microorganisms were recognized. These organisms may have some role in periodontal disease. This study reveals some potential for a mol.-biol. technique to analyze the oral microflora assocd. with periodontal disease, including previously uncharacterized and uncultured microorganisms, without cultivation.

RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB The oral bacterial flora in the saliva from two patients with periodontitis and from a periodontally healthy subject were compared using a sequence anal. of 16S rDNA libraries without cultivation. 16S rDNAs were amplified from salivary DNA by **PCR** and cloned. Randomly selected clones were partially sequenced. On the basis of sequence similarities, the clones were classified into several clusters corresponding to the major phylum of the domain Bacteria. The major phylum in the libraries was the low G + C Gram-pos. bacteria. There was no clonal sequence affiliated with periodontopathic bacteria in the salivary sample from the healthy subject, while a no. of periodontal pathogens such as Campylobacter rectus, Prevotella intermedia, Porphyromonas gingivalis and Treponema socranskii were detected in the salivary samples from the patients with periodontitis. In addn., a no. of previously uncharacterized and uncultured microorganisms were recognized. These organisms may have some role in periodontal disease. This study reveals some potential for a mol.-biol. technique to analyze the oral microflora assocd. with periodontal disease, including previously uncharacterized and uncultured microorganisms, without cultivation.

IT Campylobacter rectus
DNA sequences
Oral bacteria
Porphyromonas gingivalis
Prevotella intermedia
Treponema socranskii
(comparison of oral **bacterial flora** in saliva from a healthy subject and two human periodontitis patients by anal. of 16S rDNA sequences)

IT **DNA**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(rDNA; comparison of oral **bacterial flora** in saliva from a healthy subject and two human periodontitis patients by anal. of 16S rDNA sequences)

L5 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS
AN 1998:45922 CAPLUS
DN 128:138520
TI Characterization of the bacterial flora of Sudanese sorghum flour and sorghum sourdough
AU Hamad, S. H.; Dieng, M. C.; Ehrmann, M. A.; Vogel, R. F.
CS Lehrstuhl fur Technische Mikrobiologie, Technische Universitat Munchen, Freising-Weihenstephan, Germany
SO Journal of Applied Microbiology (1997), 83(6), 764-770
CODEN: JAMIFK; ISSN: 1364-5072
PB Blackwell Science Ltd.
DT Journal
LA English
AB Sudanese sorghum flour, a spontaneously fermented sourdough and a long-term sourdough produced in a Sudanese household by consecutive re-inoculations, was studied. The dominant contaminants of sorghum flour were Gram-neg., catalase-pos., rod-shaped bacteria with counts of about 105 cfu g-1. The spontaneously fermented sorghum sourdough showed a bacterial succession from Gram-neg., catalase-pos. contaminants to

Enterococcus faecalis, Lactococcus lactis, Lactobacillus fermentum and L. reuteri. The total bacterial count reached about 1010 cfu g-1 and the pH dropped from 6.cntdot.4 to 3.cntdot.35 in about 42 h. In this phase, only the latter two species remained dominant in a ratio of 1:1. From the Sudanese long-term dough, seven strains of Lactobacillus were isolated, representing the dominant flora. Sequence comparison of partial 16S rRNA gene sequences were used to clarify their phylogenetic positions. Five strains were classified as L. vaginalis and could be regarded as heterogeneous biovars of this species. The other two strains could be assigned to L. helveticus. RAPD-**PCR** and sugar fermn. patterns were useful in differentiation of these strains.

AB Sudanese sorghum flour, a spontaneously fermented sourdough and a long-term sourdough produced in a Sudanese household by consecutive re-inoculations, was studied. The dominant contaminants of sorghum flour were Gram-neg., catalase-pos., rod-shaped bacteria with counts of about 105 cfu g-1. The spontaneously fermented sorghum sourdough showed a bacterial succession from Gram-neg., catalase-pos. contaminants to Enterococcus faecalis, Lactococcus lactis, Lactobacillus fermentum and L. reuteri. The total bacterial count reached about 1010 cfu g-1 and the pH dropped from 6.cntdot.4 to 3.cntdot.35 in about 42 h. In this phase, only the latter two species remained dominant in a ratio of 1:1. From the Sudanese long-term dough, seven strains of Lactobacillus were isolated, representing the dominant flora. Sequence comparison of partial 16S rRNA gene sequences were used to clarify their phylogenetic positions. Five strains were classified as L. vaginalis and could be regarded as heterogeneous biovars of this species. The other two strains could be assigned to L. helveticus. RAPD-**PCR** and sugar fermn. patterns were useful in differentiation of these strains.

IT RAPD analysis
(-**PCR**; characterization of the bacterial flora of Sudanese sorghum flour and sorghum sourdough)

IT **PCR** (polymerase chain reaction)
(-RAPD; characterization of the bacterial flora of Sudanese sorghum flour and sorghum sourdough)

IT **DNA** sequences
(of 16S rRNA genes of the **bacterial flora** of Sudanese sorghum flour and sorghum sourdough)

=>

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=> s bacter### flora
L6         7538 BACTER### FLORA

=> s l6 and PCR
L7           80 L6 AND PCR

=> s l7 and eletrophoresis
L8            0 L7 AND ELETROPHORESIS

=> s l7 and electrophoresis
L9           21 L7 AND ELECTROPHORESIS

=> s l9 and probe#
L10           4 L9 AND PROBE#

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11            3 DUP REM L10 (1 DUPLICATE REMOVED)

=> d l11 1-3 bib ab kwic

L11  ANSWER 1 OF 3  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN   2002:608602  BIOSIS
DN   PREV200200608602
TI   Effects of a controlled diet and black tea drinking on the fecal flora of
     human volunteers in a double-blind randomized feeding study.
AU   Mai, V. (1); Katki, H. (1); Clevidence, B.; Harmsen, H.; Schatzkin, A. (1)
CS   (1) National Cancer Institute, Bethesda, MD USA
SO   Abstracts of the General Meeting of the American Society for Microbiology,
     (2002) Vol. 102, pp. 364-365. http://www.asmusa.org/mtgsrc/generalmeeting.
     htm. print.
     Meeting Info.: 102nd General Meeting of the American Society for
     Microbiology Salt Lake City, UT, USA May 19-23, 2002 American Society for
     Microbiology
     . ISSN: 1060-2011.
DT   Conference
LA   English
AB   The importance of the microbial intestinal flora in human health/disease
     including cancer has long been postulated and the effects of some dietary
     substances, such as prebiotics, on the flora composition have been
     established. The effects of diet on health might be partially mediated by
     changes in the composition of the intestinal flora. Polyphenols in tea
     could result in changes of the bacterial flora leading
     to an increased excretion of fecal bile acids and a reduction of serum
     lipids. We analyzed changes in the fecal flora composition of 15 subjects
     in a human feeding study that investigated the effects of black tea
     drinking on blood lipids in hypercholesterolemic volunteers. Subjects on a
     controlled diet were randomly assigned the order of tea and placebo
     drinking for two 21 day periods. The two intervention periods were
     interrupted by a 4-week washout. Fecal samples were collected on day 1,
     day 13 and day 20 of both intervention periods for a total of six samples
     from each volunteer. A small proportion of each fecal sample was fixed in
     paraformaldehyde for fluorescent in situ hybridizations (FISH) and the
     rest was frozen at -70degreeC. DNA was extracted from the frozen samples
     and the V6 to V8 region of the bacterial 16S rDNA was amplified by
     PCR for Temporal Gradient Gel Electrophoresis. FISH
     analyses with seven different probes from a subset of 8 subjects
     indicate a large intra- and interindividual variability of the fecal flora
     that masks any possible effect of tea drinking. Tea drinking did not
     consistently affect the amounts of the seven bacterial groups that were
     analyzed with specific probes. However, our analyses revealed
     that even though tea did not affect the specific bacterial groups that
     were analyzed, it did decrease the amounts of bacteria that were detected
     by the universal bacterial probe, but not by any of the specific
```

probes. Preliminary TGGE analyses showed that the profiles for each subject vary less than expected from the FISH studies. These results indicate that tea drinking affects some flora components, but more sensitive tools and larger studies are needed to evaluate effects of diet on the intestinal flora.

AB. . . partially mediated by changes in the composition of the intestinal flora. Polyphenols in tea could result in changes of the **bacterial flora** leading to an increased excretion of fecal bile acids and a reduction of serum lipids. We analyzed changes in the. . . was extracted from the frozen samples and the V6 to V8 region of the bacterial 16S rDNA was amplified by **PCR** for Temporal Gradient Gel **Electrophoresis**. FISH analyses with seven different **probes** from a subset of 8 subjects indicate a large intra- and interindividual variability of the fecal flora that masks any. . . tea drinking. Tea drinking did not consistently affect the amounts of the seven bacterial groups that were analyzed with specific **probes**. However, our analyses revealed that even though tea did not affect the specific bacterial groups that were analyzed, it did decrease the amounts of bacteria that were detected by the universal bacterial **probe**, but not by any of the specific **probes**. Preliminary TGGE analyses showed that the profiles for each subject vary less than expected from the FISH studies. These results. . .

L11 ANSWER 2 OF 3 MEDLINE DUPLICATE 1
AN 2002111595 MEDLINE
DN 21831570 PubMed ID: 11843027
TI Identification of Helicobacter pylori DNA in human cholesterol gallstones.
AU Monstein H J; Jonsson Y; Zdolsek J; Svanvik J
CS Molecular Biology Laboratory-LMO, University Hospital, Linkoping, Sweden.. hanmo@ibk.liu.se
SO SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY, (2002 Jan) 37 (1) 112-9. Journal code: 0060105. ISSN: 0036-5521.
CY Norway
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200207
ED Entered STN: 20020215
Last Updated on STN: 20020727
Entered Medline: 20020726
AB BACKGROUND: The gallbladder mucosa secretes hydrogen ions and is covered by mucus. The environmental conditions for bacterial colonization are similar to those in the stomach. Gallbladder stones often contain DNA from enteric bacteria, but no compelling evidence demonstrates that Helicobacter spp. have been present. The aim of this study was to establish bacterial DNA profiles in cholesterol gallstones with special reference to Helicobacter pylori. METHODS: Cholesterol gallstones from 20 patients were subjected to polymerase chain reaction, bacterial profiling by temporal temperature gradient gel **electrophoresis**, automated DNA sequencing, and Southern blot analysis using a Helicobacter sp. specific primer. A nested ureI-**PCR** assay was used to discriminate between gastric and non-gastric H. pylori. RESULTS: TTGE, partial 16S rDNA sequencing, and hybridization analysis revealed the presence of DNA presumably representing a mixed **bacterial flora** in cholesterol gallstones, including H. pylori in the gallstone centres in 11 out of 20 patients. In three cases, the urel-**PCR** assay revealed non-gastric H. pylori. CONCLUSIONS: These data support the presence of DNA from a mixed bacterial population, including H. pylori in cholesterol gallstones, reflecting either that H. pylori is an indigenous part of a flora in the stone-containing gallbladder or, alternatively, that H. pylori colonization in the biliary tract predisposes to cholesterol gallstone formation.

AB . . . pylori. METHODS: Cholesterol gallstones from 20 patients were subjected to polymerase chain reaction, bacterial profiling by temporal

temperature gradient gel **electrophoresis**, automated DNA sequencing, and Southern blot analysis using a Helicobacter sp. specific primer. A nested ureI-**PCR** assay was used to discriminate between gastric and non-gastric H. pylori. RESULTS: TTGE, partial 16S rDNA sequencing, and hybridization analysis revealed the presence of DNA presumably representing a mixed **bacterial flora** in cholesterol gallstones, including H. pylori in the gallstone centres in 11 out of 20 patients. In three cases, the urel-**PCR** assay revealed non-gastric H. pylori. CONCLUSIONS: These data support the presence of DNA from a mixed bacterial population, including H.. . .

CT . . . Gov't
Adult
Aged
*Cholelithiasis: GE, genetics
*Cholelithiasis: MI, microbiology
*Cholesterol: GE, genetics
*Cholesterol: IP, isolation & purification
Colony Count, Microbial
DNA Probes: GE, genetics
*DNA, Bacterial: GE, genetics
*DNA, Bacterial: IP, isolation & purification
*Helicobacter pylori: GE, genetics
*Helicobacter pylori:. . .

CN 0 (DNA **Probes**); 0 (DNA, Bacterial)

L11 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:201620 BIOSIS
DN PREV200200201620
TI Characterization of microbial communities from a sulfate-reducing fluidized bed reactor using fluorescent in situ hybridization (FISH) and denaturing gradient gel **electrophoresis** (DGGE.
AU Maukonen, J. M. (1); Ratto, M. (1)
CS (1) VTT Biotechnology, Espoo Finland
SO Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 601-602. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DT Conference
LA English
AB Background: The application of molecular biological techniques offers new opportunities for the analysis of microbial communities. Denaturing gradient gel **electrophoresis** (DGGE) and fluorescent in situ hybridization (FISH) have, therefore, become very powerful molecular tools in environmental microbiology. This study focuses on the development of methods for characterization of microbial flora and dynamics in sulfate-reducing reactors. Methods: The bacterial samples were taken from a fluidized bed reactor, which is used for treatment of metal-containing wastewater. The total DNA was extracted from the samples and used as a template in the **PCR**. The primers used generate a DNA fragment of about 200 base-pairs. The fragment codes for all eubacterial 16S rRNA. The samples for FISH were fixed and immobilized on gelatin-coated slides. Several fluorescein-labeled **probes**, both eubacterial and SRB-specific, were tested. The FISH samples were examined with a BX60-epifluorescent microscope. Results: The DGGE-results showed that there were many bacterial strains present in the reactor. It can also be seen from the results that a few dominating bacterial strains follow through the tested time period. According to the results, the DGGE was able to show the decline in bacterial diversity when process failures occur. After re-inoculation into the reactor, the original bacterial populations were mainly restored. The FISH results showed that the **bacterial flora** in the reactor was rather diverse.
Conclusion: The advantages of molecular methods in microbial ecology are

quite obvious. Oligonucleotide **probes** provide means to rapidly identify members of SRB, in both pure cultures and environmental samples. In particular, rRNA-directed oligonucleotides designed for specific groups of SRB have been demonstrated to represent valuable tools for group- and species-specific hybridization studies in complex communities without prior isolation of the target organism. According to this study the combination of DGGE and FISH provides insight into the microbial community structure and the dynamics of the **bacterial flora** of a fluidized bed reactor.

TI . . . Characterization of microbial communities from a sulfate-reducing fluidized bed reactor using fluorescent in situ hybridization (FISH) and denaturing gradient gel **electrophoresis** (DGGE.

AB Background: The application of molecular biological techniques offers new opportunities for the analysis of microbial communities. Denaturing gradient gel **electrophoresis** (DGGE) and fluorescent in situ hybridization (FISH) have, therefore, become very powerful molecular tools in environmental microbiology. This study focuses. . . for treatment of metal-containing wastewater. The total DNA was extracted from the samples and used as a template in the **PCR**. The primers used generate a DNA fragment of about 200 base-pairs. The fragment codes for all eubacterial 16S rRNA. The samples for FISH were fixed and immobilized on gelatin-coated slides. Several fluorescein-labeled **probes**, both eubacterial and SRB-specific, were tested. The FISH samples were examined with a BX60-epifluorescent microscope. Results: The DGGE-results showed that. . . failures occur. After re-inoculation into the reactor, the original bacterial populations were mainly restored. The FISH results showed that the **bacterial flora** in the reactor was rather diverse. Conclusion: The advantages of molecular methods in microbial ecology are quite obvious. Oligonucleotide **probes** provide means to rapidly identify members of SRB, in both pure cultures and environmental samples. In particular, rRNA-directed oligonucleotides designed. . . this study the combination of DGGE and FISH provides insight into the microbial community structure and the dynamics of the **bacterial flora** of a fluidized bed reactor.

IT Methods & Equipment
bioreactors: equipment, uses; denaturing gradient gel **electrophoresis**: analytical method, applications; fluidized bed reactors: equipment, uses; fluorescence in-situ hybridization: analytical method, applications

IT Miscellaneous Descriptors
environmental microbiology; methodology;. . .

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L12 ANSWER 1 OF 3 MEDLINE
AN 2003147330 IN-PROCESS
DN 22549487 PubMed ID: 12662383
TI Profiling and Identification of Eubacteria in the Stomach of Mongolian Gerbils With and Without Helicobacter pylori Infection.
AU Sun Yi-Qian; Monstein Hans-Jurg; Nilsson Lennart E; Petersson Fredrik; Borch Kurt
CS Division of Surgery, Department of Biomedicine and Surgery; Molecular Biology Laboratory - LMO; Clinical Microbiology - IMK; Faculty of Health Sciences, University of Linkoping, and Pathology Research Department, Ryhov Hospital, Jonkoping, Sweden.
SO HELICOBACTER, (2003 Apr) 8 (2) 149-57.
Journal code: 9605411. ISSN: 1083-4389.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20030331
Last Updated on STN: 20030331
AB BACKGROUND: Mongolian gerbils are frequently used to study Helicobacter pylori-induced gastritis and its consequences. The presence of an indigenous bacterial flora with suppressive effect on H. pylori may cause difficulties with establishing this experimental model. AIM: The aim of the present study was to determine bacterial profiles in the stomach of Mongolian gerbils with and without (controls) H. pylori infection. METHODS: Gastric tissue from H. pylori ATCC 43504 and CCUG 17874 infected and control animals were subjected to microbial culturing and histology. In addition, gastric mucosal samples from H. pylori ATCC 43504 infected and control animals were analyzed for bacterial profiling by temporal temperature gradient gel **electrophoresis** (TTGE), cloning and pyrosequencing of 16S rDNA variable V3 region derived **PCR** amplicons. RESULTS: Oral administration of H. pylori ATCC 43504, but not CCUG 17874, induced colonization and gastric inflammation in the stomach of Mongolian gerbils. Temporal temperature gradient gel **electrophoresis** (TTGE) and partial 16S rDNA pyrosequencing revealed the presence of **DNA** representing a mixed **bacterial flora** in the stomach of both H. pylori ATCC 43504 infected and control animals. In both cases, lactobacilli appeared to be dominant. CONCLUSION: These findings suggest that indigenous bacteria, particularly lactobacilli, may have an impact on the colonization and growth of H. pylori strains in the stomach of Mongolian gerbils.
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L12 ANSWER 2 OF 3 MEDLINE
AN 2003059332 MEDLINE
DN 22423434 PubMed ID: 12534811
TI Employment of broad-range 16S rRNA **PCR** to detect aetiological agents of infection from clinical specimens in patients with acute meningitis--rapid separation of 16S rRNA **PCR** amplicons without the need for cloning.
AU Xu J; Millar B C; Moore J E; Murphy K; Webb H; Fox A J; Cafferkey M; Crowe M J
CS Northern Ireland Public Health Laboratory, Department of Bacteriology,

Belfast City Hospital, Belfast, UK.
SO JOURNAL OF APPLIED MICROBIOLOGY, (2003) 94 (2) 197-206.
Journal code: 9706280. ISSN: 1364-5072.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200303
ED Entered STN: 20030207
Last Updated on STN: 20030307
Entered Medline: 20030306
AB AIMS: The aim of this study was to develop a polyacrylamide gel **electrophoresis** (PAGE) method for the rapid separation of 16S rRNA **PCR** amplicons from aetiological agents of acute meningitis.
METHODS AND RESULTS: Blood samples from 40 patients with suspected acute meningococcal meningitis were examined for the presence of causal agents, including Neisseria meningitidis employing two methods: (i) broad-range 16S rRNA **PCR** in conjunction with PAGE and automated sequencing and (ii) species-specific **PCR** employing ABI TaqMan technology for N. meningitidis. Analysis of clinical specimens employing 16S rRNA **PCR** yielded 33/40 (82.5%) positive for the presence of bacterial DNA. Species-specific **PCR** yielded 30/40 (75%) clinical specimens positive for N. meningitidis. Prior to separation by PAGE, only 6/33 (18.2%) amplicons were able to be identified by sequence analysis, the remaining amplicons (n=27) did not yield an identification due to the presence of mixed 16S rRNA **PCR** amplicons. Following separation, amplicons were re-amplified and sequenced, yielding 24/27 (88.9%) positive for N. meningitidis and three specimens positive for Acinetobacter sp., Staphylococcus aureus and Streptococcus pneumoniae. One specimen was positive for both N. meningitidis and Streptococcus spp. and another specimen was positive for N. meningitidis and Pseudomonas sp., by broad-range **PCR**. Seven clinical specimens were negative for N. meningitidis and other eubacteria using both detection techniques.
CONCLUSIONS: Clinical specimens including blood and cerebrospinal fluid from patients with suspected acute **bacterial** meningitis, may become contaminated with commensal skin **flora**, resulting in difficulties in downstream sequencing of pathogen plus contaminant **DNA**. This study allows for the rapid separation of amplified pathogen from contaminant DNA. SIGNIFICANCE AND IMPACT OF STUDY: This study demonstrated the usefulness of the rapid separation of multiple 16S rRNA **PCR** amplicons using a combination of PAGE and automated sequencing, without the need of cloning. Adoption of this technique is therefore proposed when trying to rapidly identify pathogens in clinical specimens employing broad-range 16S rRNA **PCR**.
TI Employment of broad-range 16S rRNA **PCR** to detect aetiological agents of infection from clinical specimens in patients with acute meningitis--rapid separation of 16S rRNA **PCR** amplicons without the need for cloning.
AB AIMS: The aim of this study was to develop a polyacrylamide gel **electrophoresis** (PAGE) method for the rapid separation of 16S rRNA **PCR** amplicons from aetiological agents of acute meningitis.
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meningitidis and three specimens positive for. . . for both N. meningitidis and Streptococcus spp. and another specimen was positive for N. meningitidis and Pseudomonas sp., by broad-range **PCR**. Seven clinical specimens were negative for N. meningitidis and other eubacteria using both detection techniques. CONCLUSIONS: Clinical specimens including blood and cerebrospinal fluid from patients with suspected acute **bacterial** meningitis, may become contaminated with commensal skin **flora**, resulting in difficulties in downstream sequencing of pathogen plus contaminant **DNA**. This study allows for the rapid separation of amplified pathogen from contaminant DNA. SIGNIFICANCE AND IMPACT OF STUDY: This study demonstrated the usefulness of the rapid separation of multiple 16S rRNA **PCR** amplicons using a combination of PAGE and automated sequencing, without the need of cloning. Adoption of this technique is therefore proposed when trying to rapidly identify pathogens in clinical specimens employing broad-range 16S rRNA **PCR**.

CT . . . Check Tags: Human; Support, Non-U.S. Gov't
Acinetobacter: IP, isolation & purification
Acute Disease
Child
Child, Preschool
DNA, Bacterial: AN, analysis
Electrophoresis, Polyacrylamide Gel: MT, methods
Infant
*Meningitis: MI, microbiology
Neisseria meningitidis: IP, isolation & purification
*Polymerase Chain Reaction: MT, methods

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L12 ANSWER 3 OF 3 MEDLINE
AN 2002111595 MEDLINE
DN 21831570 PubMed ID: 11843027
TI Identification of Helicobacter pylori DNA in human cholesterol gallstones.
AU Monstein H J; Jonsson Y; Zdolsek J; Svanvik J
CS Molecular Biology Laboratory-LMO, University Hospital, Linkoping, Sweden.. hanmo@ibk.liu.se
SO SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY, (2002 Jan) 37 (1) 112-9. Journal code: 0060105. ISSN: 0036-5521.
CY Norway
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200207
ED Entered STN: 20020215
Last Updated on STN: 20020727
Entered Medline: 20020726
AB BACKGROUND: The gallbladder mucosa secretes hydrogen ions and is covered by mucus. The environmental conditions for bacterial colonization are similar to those in the stomach. Gallbladder stones often contain DNA from enteric bacteria, but no compelling evidence demonstrates that Helicobacter spp. have been present. The aim of this study was to establish bacterial DNA profiles in cholesterol gallstones with special reference to Helicobacter pylori. METHODS: Cholesterol gallstones from 20 patients were subjected to polymerase chain reaction, bacterial profiling by temporal temperature gradient gel **electrophoresis**, automated DNA sequencing, and Southern blot analysis using a Helicobacter sp. specific primer. A nested ureI-**PCR** assay was used to discriminate between gastric and non-gastric H. pylori. RESULTS: TTGE, partial 16S rDNA sequencing, and hybridization analysis revealed the presence of **DNA** presumably representing a mixed **bacterial flora** in cholesterol gallstones, including H.

pylori in the gallstone centres in 11 out of 20 patients. In three cases, the urel-**PCR** assay revealed non-gastric H. pylori. CONCLUSIONS: These data support the presence of DNA from a mixed bacterial population, including H. pylori in cholesterol gallstones, reflecting either that H. pylori is an indigenous part of a flora in the stone-containing gallbladder or, alternatively, that H. pylori colonization in the biliary tract predisposes to cholesterol gallstone formation.

AB . . . pylori. METHODS: Cholesterol gallstones from 20 patients were subjected to polymerase chain reaction, bacterial profiling by temporal temperature gradient gel **electrophoresis**, automated DNA sequencing, and Southern blot analysis using a Helicobacter sp. specific primer. A nested ureI-**PCR** assay was used to discriminate between gastric and non-gastric H. pylori. RESULTS: TTGE, partial 16S rDNA sequencing, and hybridization analysis revealed the presence of **DNA** presumably representing a mixed **bacterial flora** in cholesterol gallstones, including H. pylori in the gallstone centres in 11 out of 20 patients. In three cases, the urel-**PCR** assay revealed non-gastric H. pylori. CONCLUSIONS: These data support the presence of DNA from a mixed bacterial population, including H.. . .

100697

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DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L14	L13 and chip$1	11	L14
L13	(apparatus or device) near5 (pcr or amplification chain reaction$1) near5 (electrophore$3 near5 analy$3 or detect$3)	40	L13
L12	l9 and intestin$2	2	L12
L11	l9 and (intestin$2 near5 flora)	0	L11
L10	L9 and (intestinal near5 flora)	0	L10
L9	l7 and bacter$3	42	L9
L8	L7 and (intestinal near5 bacter$3)	0	L8
L7	l5 and (hybridiz$5 near5 probe$1)	53	L7
L6	L5 and hybridiz$5	81	L6
L5	L4 and (analyzer or detector)	103	L5
L4	L3 and electrophore$3	278	L4
L3	PCR near5 (apparatus$2 or device$1)	539	L3
L2	l1 and PCR	0	L2
L1	(apparatus$2 or device$1) near5 amplif$3 near electrophore$3 near5 (detect$2 or analyz$2)	2	L1

END OF SEARCH HISTORY

☐ Generate Collection

L14: Entry 3 of 11 File: USPT Jul 2, 2002

DOCUMENT-IDENTIFIER: US 6413766 B2
TITLE: Rapid thermocycling for sample analysis

Brief Summary Text (19):
U.S. Pat. No. 5,498,392 discloses chip-like devices for amplifying a preselected polynucleotide in a sample by conducting a polynucleotide polymerization reaction. The devices comprise a substrate microfabricated to define a sample inlet port and a mesoscale flow system, which extends from the inlet port. A polynucleotide polymerization reaction chamber containing reagents for polymerization and amplification of a polynucleotide is in fluid communication with the inlet port. A heat source and, optionally, a cooling source are used to heat and/or cool the chip.

Brief Summary Text (20):
Wilding and co-workers, Nucleic Acids Res., 24:380-385 (1996), demonstrated that PCR could be carried out in a microfabricated silicon glass chip-like chamber. By contacting enclosed 12 microliter reaction chambers microfabricated in glass to a block heater which cycled between two temperatures, they were able to obtain effective and reproducible PCR amplification, as confirmed by removing the PCR product and evaluating it using capillary electrophoresis. Similarly, Northrup and co-workers, Anal. Chem., 68:4081-4086 (1996), accomplished PCR amplification of DNA in a microfabricated silicon PCR device that could be directly interfaced with an electrophoretic chip for PCR product analysis. The device contained disposable polypropylene liners to retain the PCR mixture which could be cycled between two temperatures using polysilicon heaters in direct contact with the PCR chamber and cooled either passively or by air drawn along the heater surfaces of the reaction chamber. The device was interfaced with the electrophoretic chip by forcing it into the 1 mm drilled holes in the electrophoretic chip.

Brief Summary Text (25):
The possibilities of thermocycling on a device in which thermocycling is achieved using a heating and/or cooling element are predetermined by the initial design of the chip, as the location of the heating and/or cooling element is typically part of the chip itself. Thus, these microdevices use of thermocycling is spatially constrained and the devices are not flexible with respect to the use of heating or cooling on different locations within or at the microdevice structure.

Detailed Description Text (13):
Another preferred example of a suitable reaction vessel is the channel structure incorporated into a microfabricated device, such as the microfabricated substrate described by Wilding and co-workers in Nucleic Acids Res., 24:380-385 (1996). Other reaction vessels with characteristics suitable for rapid thermocycling are shown in FIGS. 6A-6D, comprising an entrenched reservoir attached or incorporated into a microfabricated chip device.

Detailed Description Text (14):
One preferred embodiment of a microchip for use in the present invention is illustrated in FIGS. 1A, 1B, 1C and 1D. It will be appreciated that FIGS. 1A-1D depict only a portion of the chip, and that the depiction is not drawn to scale. FIG. 1A depicts a corner of a microchip 2 having a glass upper portion or layer 4 and a glass lower portion or layer 6 wherein the upper and lower layers fit together, for example, through bonding by high temperature. Upper layer 4 contains an upwardly open sample reservoir 8 and an upwardly open waste reservoir 10. A "window" 12 provided in upper layer 4 allows for direct heating of the sample by an IR source. It will be understood that window 12 can be provided in a number of ways. For example, the "window" can merely be a portion of the upper layer 4 of substantially full thickness of the upper layer 4, or can be an area which has been made thinner with respect to adjacent

portions of the upper layer 4 by means known in the art, such as etching. A window 12 is more clearly depicted in FIG. 1D, which is a cross section view taken along line 1-D of FIG. 1A when the upper layer 4 and lower layer 6 are fitted together. Returning to FIG. 1A, a microchannel 14 is provided in the lower layer 6, and the area where a PCR reaction occurs is generally depicted by the elliptical area 16. FIG. 1B shows essentially the same portion of a microchip as shown in FIG. 1A, only having a widened microchannel 18 that has a greater volume than microchannel 16 of FIG. 1A. FIG. 1C is a top plan view of the upper layer 4 of microchip 2 of FIG. 1A, showing sample reservoir 8 and waste reservoir 10 and window 12. In FIG. 1D, upper layer 4 is shown having sample reservoir 8 and waste reservoir 10 and window 12. Lower layer 6 is shown having microchannel 14. A second microchannel 20 connects sample reservoir 8 and microchannel 14. A third microchannel 22 connects microchannel 14 and waste reservoir 10. FIGS. 1C and 1D are not drawn to scale with respect to FIG. 1A. It will be understood that the microchip shown in FIGS. 1A-1D can also be made of plastic or any other material that could be penetrated by IR light wavelengths. In such an embodiment, the upper and lower layers could be fit together, for example, by a UV curing process, and the window could be formed by electroforming during fabrication of the upper layer.

Detailed Description Text (51):
As can be seen from the figures, according to the present invention both a non-contact heat source and a non-contact cooling source are used. This allows for repeated introduction of any number of reaction vessels in and out of the apparatus. Thus, the present invention provides an economic advantage over other thermocycling apparatus, in that it is only a relatively inexpensive microchip, capillary tube, or other reaction vessel that must be changed for every sample. Some methods provided in the art require the physical attachment of the heating and/or cooling means to the reaction vessel itself. Therefore, unless the reaction vessel could be completely cleaned to ensure that contamination from one sample to another did not occur, a new chip attached to a new heating and/or cooling device would have to be provided for every sample. While for ease of reference only one sample-containing vessel was shown and/or described in these embodiments, it is equally within the scope of the invention to thermocycle two or more samples at the same time. In addition, because the heating and cooling means are relatively stationary in the apparatus of the present invention, the reaction vessel can be moved in any direction relative to the heating and/or cooling sources.

Detailed Description Text (54):
The present invention also teaches methods and apparatus for use of an IR heat source in conjunction with a microchip that allows for movement or "pumping" of the sample through the chip.

Detailed Description Text (55):
Electrophoretic "chips" consist of glass or plastic substrates into which a pattern of microchannels, often simple but sometimes complicated, have been fabricated. Embodiments of such chips are shown in FIGS. 1 and 2. The microchannels terminate at reservoirs which are often drilled holes in the structure itself and which hold volumes as low as a few microliters to as much as several tens of microliters. The flow or pumping of fluid through the microchannel architecture, that is, from one reservoir to another or through any components fabricated within the microchannels themselves, is typically carried out in one of two ways: by hydrostatic pressure or by electric field-driven flow (endosmotic flow or "EOF").

Detailed Description Text (56):
Microchannel structures on electrophoretic chips are typically in the micrometer range, with depths ranging from 10-50 .mu.m and widths from 30-100 .mu.m. A microchannel with the dimensions of 10 .mu.m (deep).times.50 .mu.m (wide).times.3 mm (long) has a total microchannel volume of 1.5 nanoliters (nL). Therefore, to flow the equivalent of 20 microchannel volumes of a given solution through this particular microchannel would require the pumping of 30 nL of solution. The controlled flow of these ultralow volumes through the microchannels of microfabricated electrophoretic devices may be difficult to regulate with hydrostatic pressure. The low volume pumping of solution on chips is more conducive to EOF which can be controlled quite accurately by the magnitude of the voltage applied. A limitation associated with EO-driven flow, however, is its dependence on two parameters: 1) the ionic strength of the

constituents of the solution being pumped; and 2) the chemical composition of the microchannel wall. For example, a low, negligible EOF is likely to result when using solutions containing a large salt concentration, such as 100 mM NaCl or 2.times. TBE solutions. Low EOF will also result when the interior channel walls are composed of a neutral or nonionic substance such as plastic. Under such conditions, EO flow is likely to be of limited use with respect to its ability to pump solution through the microchannel architecture. This limits the use of microfabricated electrophoretic devices as the basic element in the "laboratory on a chip" concept where a diverse array of biological and chemical solutions will need to be pumped effectively through the microchannel architecture.

Detailed Description Text (88):
Not only does this example demonstrate the utility of the thermocycler for another common PCR procedure, competitive PCR reactions, but the analysis of the DNA products on the short capillary approximate the migration distance and analysis times that would be observed for an "on-chip" CE separation. By integration of "on-chip" PCR with "on-chip" CE analysis, very favorable total analysis times can be attained which would greatly facilitate tedious PCR experiments such as competitive-PCR reactions which involve several PCR reactions over a wide range of starting competitor and target concentrations. The analysis times for a complete QC-PCR experiment may take days using conventional isotope-labeling, amplification and detection methodologies. The automation offered by integration of the PCR and DNA detection methods in a chip format would be a great advance for the quantification of the competitor/target DNA combinations required to plot useful data.

Other Reference Publication (2):
Cheng et al., "Chip PCR.II. Investigation of different PCR Amplification systems in microfabricated silicon-glass chips," 1996, Nucleic Acids Research, vol. 24, No. 2, pp. 380-385, USA.

Other Reference Publication (7):
Fluri, K. et al., "Integrated Capillary Electrophoresis Devices with an Efficient Postcolumn Reactor in Planar Quartz and Glass Chips," Anal. Chem. vol. 68, pp. 4285-4290, Dec. 1, 1996.

Other Reference Publication (9):
Seiler, K. et al., "Electroosmotic Pumping and Valveless Control of Fluid Flow within a Manifold of Capillaries on a Glass Chip," Anal. Chem., vol. 66, pp. 3485-3491, Oct. 15, 1994.

Other Reference Publication (11):
Shoffner, M.A. et al., "Chip PCR.I. Surface passivation of microfabricated silicon-glass chips for PCR, Nucleic Acids Research," vol. 24, No. 2, pp. 375-379, Jan. 15, 1996.

☐ Generate Collection

L14: Entry 10 of 11 File: USPT Feb 10, 1998

DOCUMENT-IDENTIFIER: US 5716825 A
TITLE: Integrated nucleic acid analysis system for MALDI-TOF MS

Brief Summary Text (6):
Genetic analysis of human disease involves large-scale detection and screening of clinical DNA samples. Current methods of DNA analysis employ conventional PCR thermal cycling devices and multiple instruments for preparing, separating, and detecting the DNA. Sample preparation is carried out with conventional sample handling devices (test tubes, pipettors, microcentrifuges, concentrators, filtration devices) and involves multiple manual handling steps and transfers. Such procedures are labor-intensive, time-consuming, costly, and susceptible to sample contamination and loss.

Detailed Description Text (4):
The term "thin film support" is used herein to refer to a substantially planar manifold made of a non-conducting material that includes a microchannel and other necessary components of a miniaturized sample preparation compartment, an interface to non-consumable parts, and an ionization surface for MALDI-TOF MS. Such a miniaturized device may be formed from a variety of materials (e.g., silicon, glass, low cost polymers) by techniques that are well-known in the art (e.g., micromachining, chemical etching, laser ablation, and the like). Portions of the device may be fabricated from composite materials. For example, a thermally insulated reaction zone may be formed from bonded layers of materials having different thermal conductivities. Established techniques exist for micromachining planar materials such as silicon and provide a useful and well-accepted approach to miniaturization. Examples of the use of such micromachining techniques to produce miniaturized separation devices on silicon or borosilicate glass chips can be found in U.S. Pat. No. 5,194,133 to Clark et al; U.S. Pat. No. 5,132,012 to Miura et al, U.S. Pat. No. 4,908,112 to Pace; and in U.S. Pat. No. 4,891,120 to Sethi et al.

Other Reference Publication (10):
Wooley and Mathies, "Ultra-high-speed DNA Fragment Separations Using Microfabricated Capillary Array Electrophoresis Chips", Proc. Natl. Acad. Sci. USA, vol. 91, pp. pp. 11348-11352 (1994).